Q: Do plants either express Snorkel or Sub1A-1?

A: Yes, plants can express both

Growth responses of seedlings under complete submergence in rice cultivars carrying both the submergence-tolerance gene *SUB1A-1* and the floating genes *SNORKELs*

Shunsuke Oe, Daisuke Sasayama, Quanshu Luo, Hiroshi Fukayama, Tomoko Hatanaka & Tetsushi Azuma

ABSTRACT

We screened 80 Asian rice (*Oryza sativa* L.) cultivars for the presence of the submergence-tolerance gene *SUB1A-1* and the floating genes *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*), and found that the deepwater rice cultivar Baisbish (BSB) and the submergence-tolerant cultivar Flood Resistant 13A (FR13A) both possess the *SUB1A-1* and the *SK1/2*. When BSB and FR13A seedlings were completely submerged, spindly growth of shoots was induced in BSB but not in FR13A. Submergence significantly increased the *SUB1A-1* transcript abundance in BSB and FR13A shoots, but the expression level in BSB was much lower than that of FR13A. Submergence also induced the expression of both *ERF66* and *ERF67*, the transcriptional targets of SUB1A-1, in FR13A shoots, whereas it upregulated the expression of *ERF67* but not that of *ERF66* in BSB shoots. These results suggest that BSB could not display submergence tolerance due to the low expression of *SUB1A-1* and/or *ERF66* under submergence.

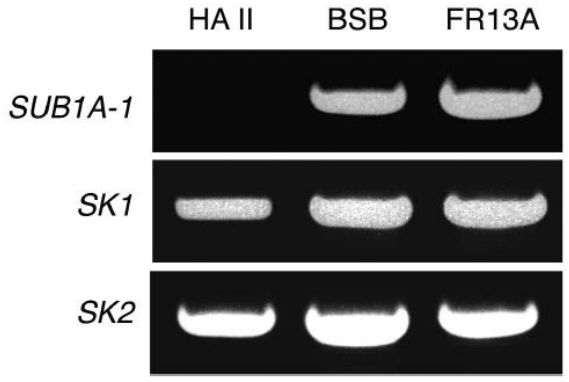
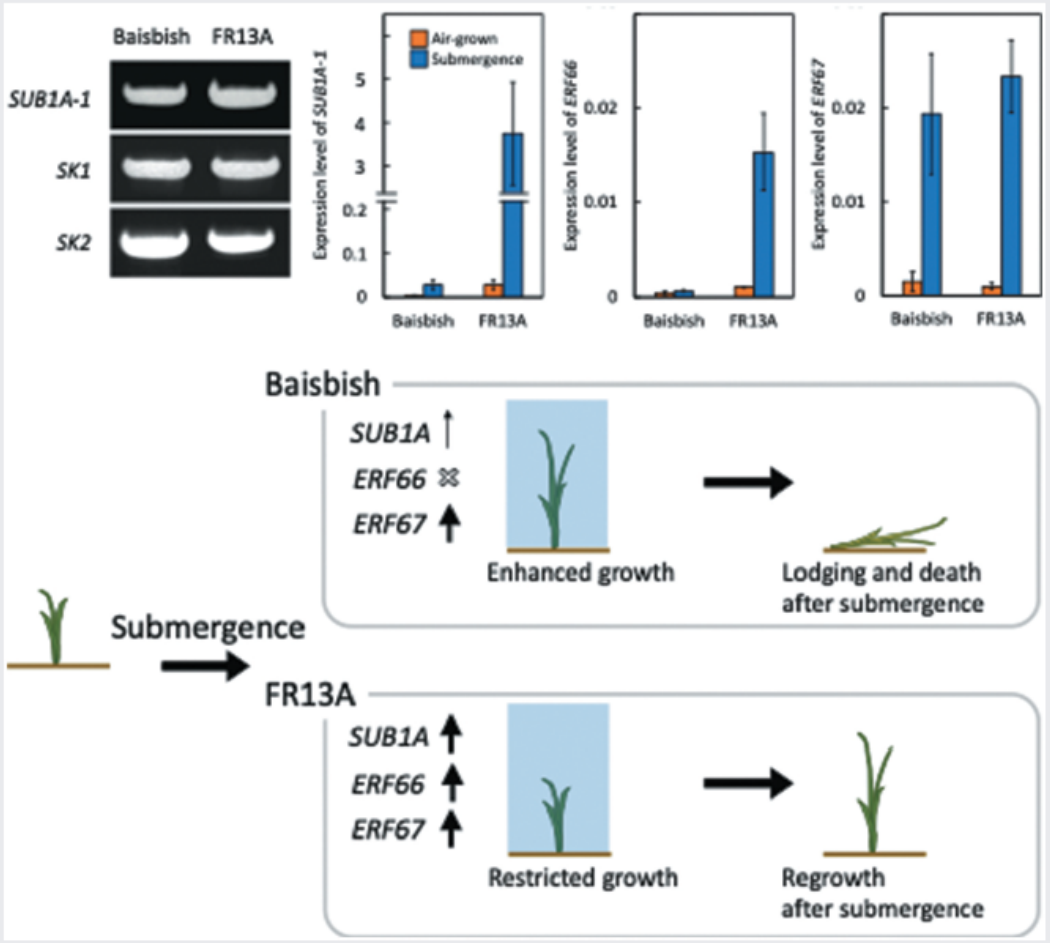
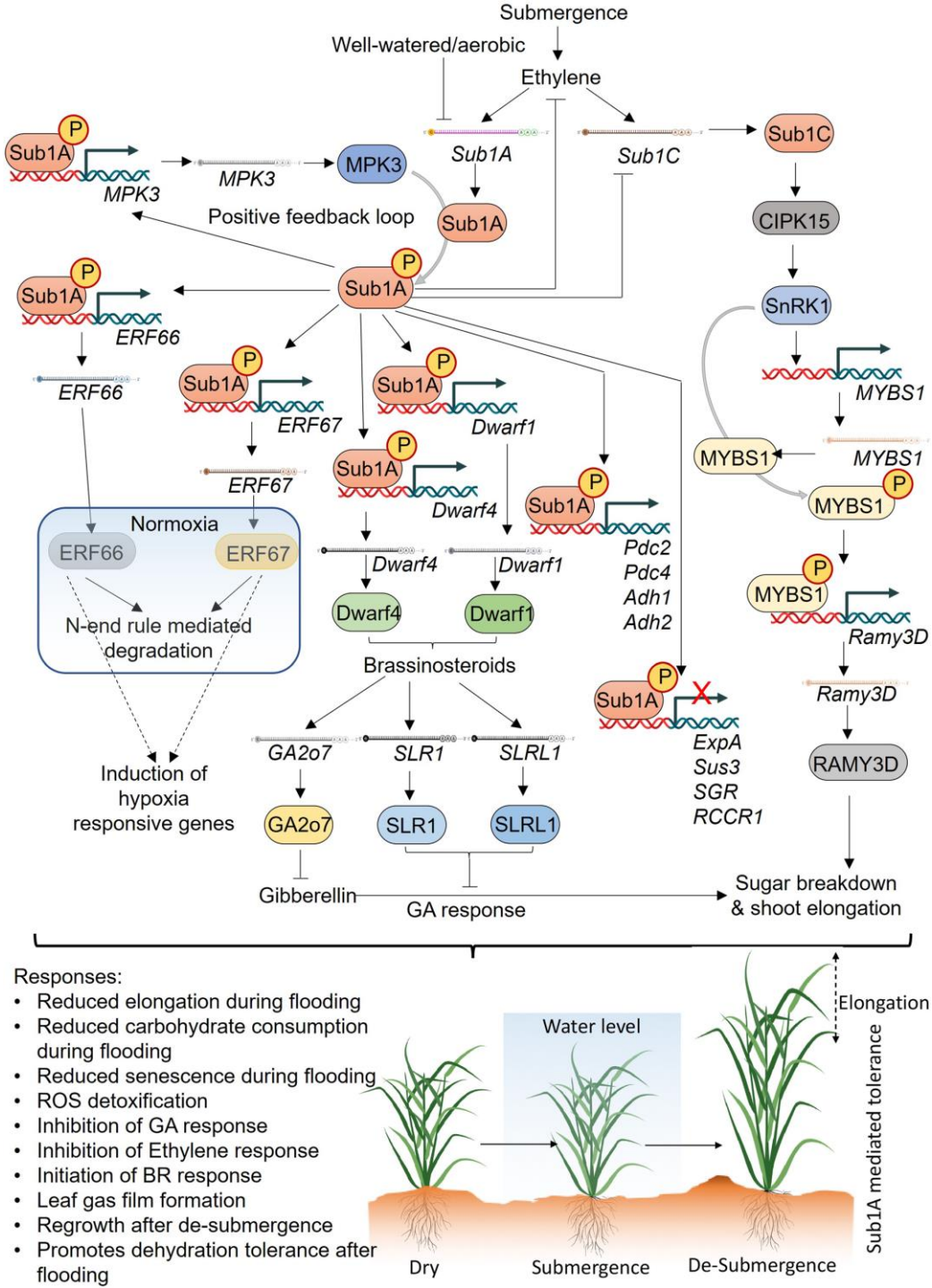


Figure 1. PCR analysis of the *SUB1A-1*, *SK1*, and *SK2* genes in the deepwater rice cultivars HA II and BSB, and the submergence-tolerant cultivar FR13A.

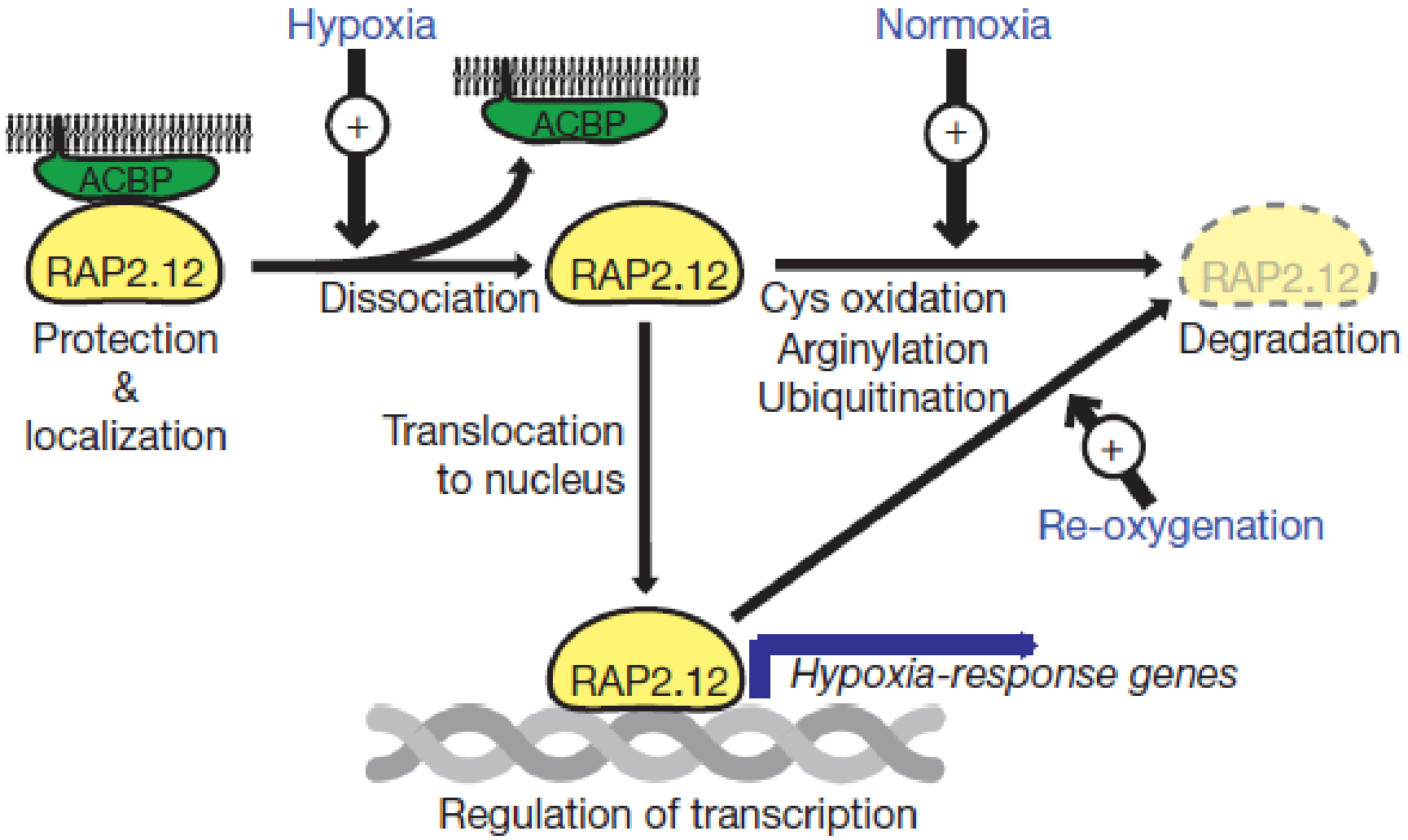


Q: Is Sub1C-1 also involved in resistance to flooding stress?

A: Yes, but Sub1A-1 plays a major role



In Arabidopsis



Model describing the oxygen sensor mechanism in plants. The transcription factor RAP2.12 is constitutively expressed under aerobic conditions. RAP2.12 protein is always present, bound to ACBP to prevent RAP2.12 frommoving into the nucleus under aerobic conditions and to protect it against proteasomal degradation in air. Upon hypoxia, RAP2.12 moves into the nucleus, where it activates anaerobic-gene expression. Upon reoxygenation, RAP2.12 is rapidly degraded via the N-end rule pathway and proteasome-mediated proteolysis to downregulate the hypoxic response.

In plants the “equivalent” transcription factors to HIF1a are the ERFVIIs.

LETTER

doi:10.1038/nature10536

Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization

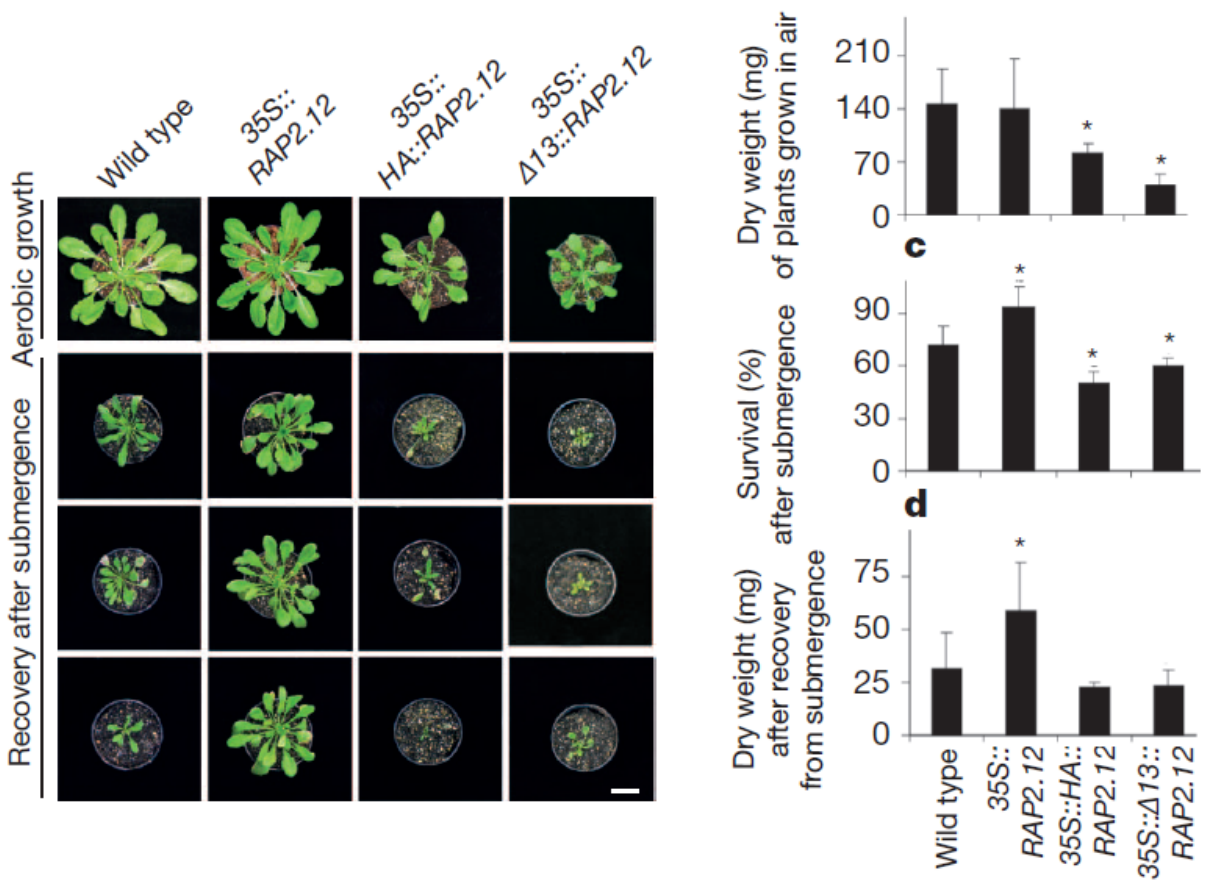
Francesco Licausi[1,2], Monika Kosmacz[1], Daan A. Weits[1], Beatrice Giuntoli[2], Federico M. Giorgi[1], Laurentius A. C. J. Voesenek[3,4], Pierdomenico Perata[2] & Joost T. van Dongen[1]

LETTER

doi:10.1038/nature10534

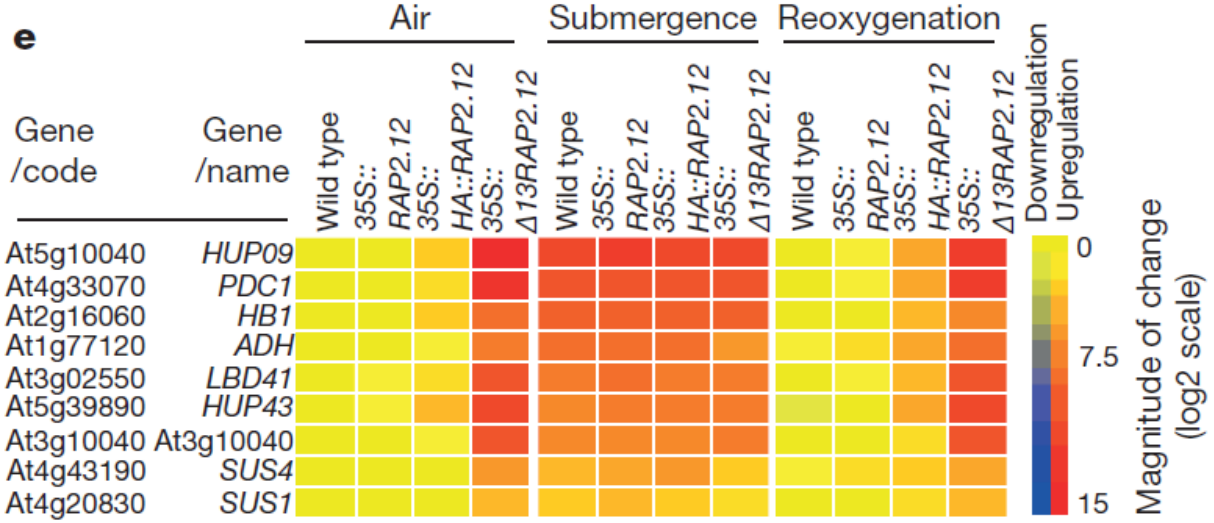
Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants

Daniel J. Gibbs[1]*, Seung Cho Lee[2]*, Nurulhikma Md Isa[1], Silvia Gramuglia[1], Takeshi Fukao[2], George W. Bassel[1], Cristina Sousa Correia[1], Françoise Corbineau[3], Frederica L. Theodoulou[4], Julia Bailey-Serres[2] & Michael J. Holdsworth[1]

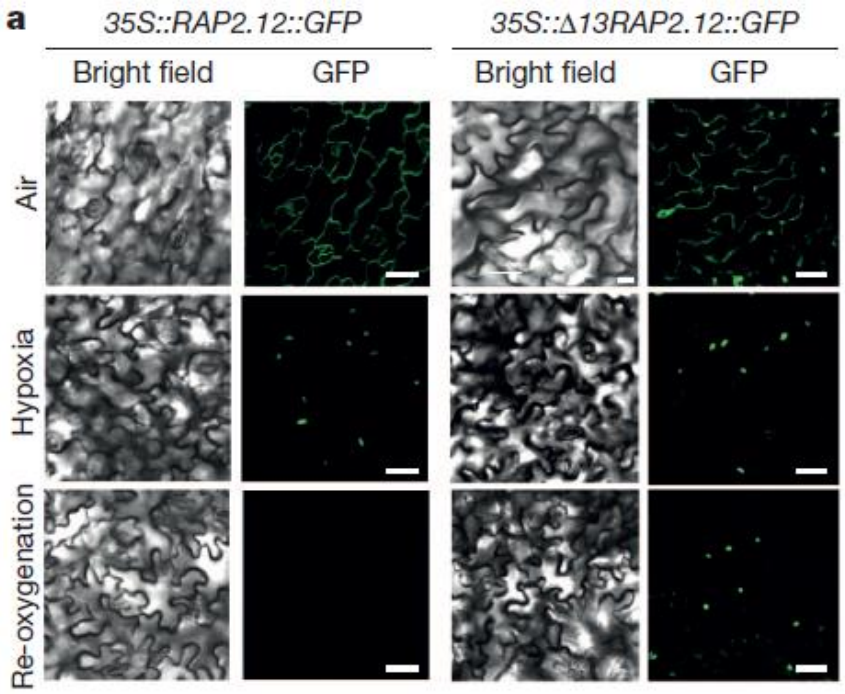


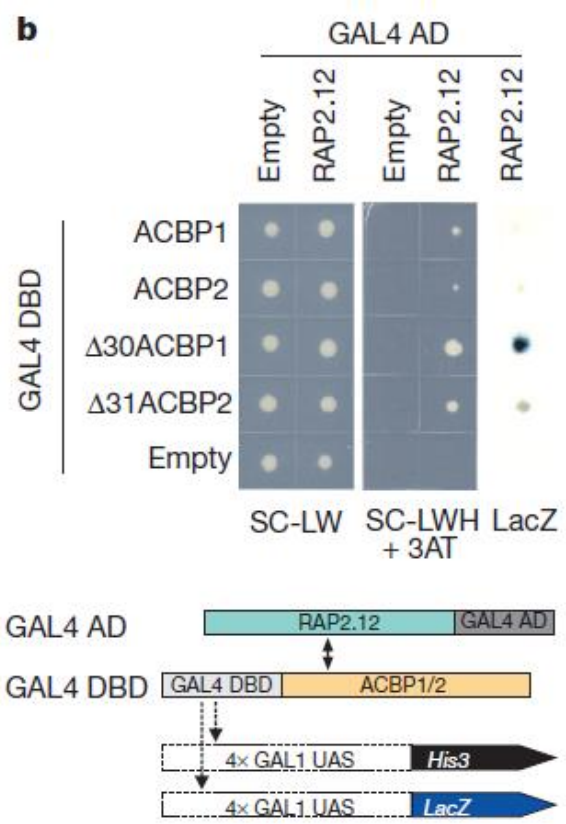
In Arabidopsis, a conserved amino-terminal amino acid sequence of the ethylene response factor (ERF)-transcription factor RAP2.12

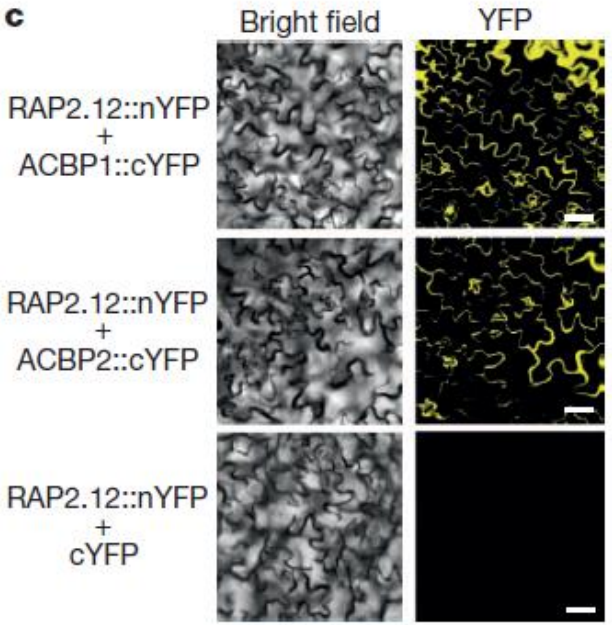
- 35S::RAP2.12 = constitutive overexpression ofRAP2.12
- 35S::HA::RAP2.12 = haemagglutinin (HA)-peptide tag at its N terminus
- RAP2.12 was expressed from which the first 13 amino acid residues were deleted (35S::Δ13RAP2.12).

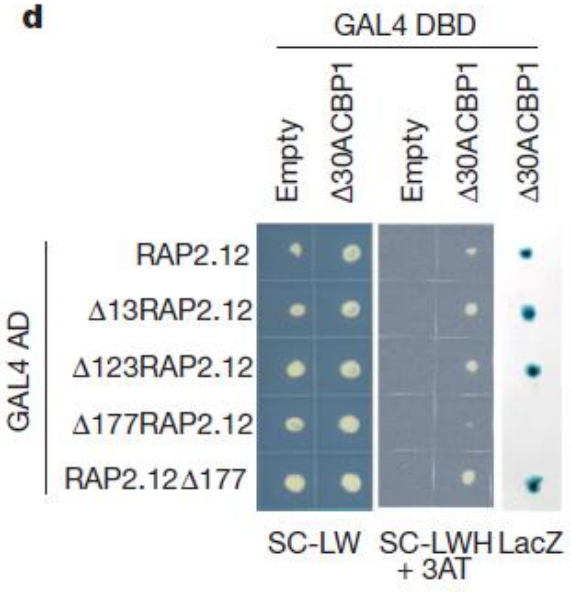


hypoxia marker genes









RAP2.12 is membrane localized and re-localizes in the nucleus upon hypoxia.

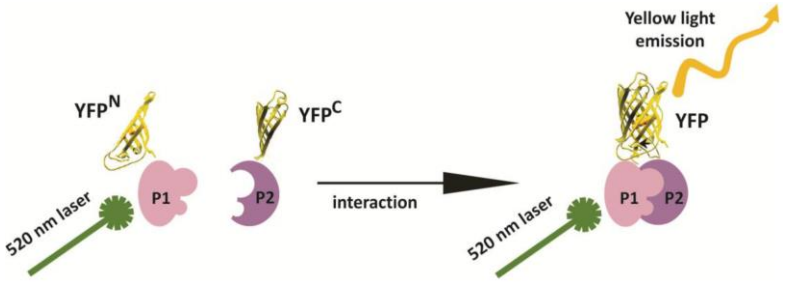
a, Subcellular localization of stably transformed GFP-fused RAP2.12 and Δ13RAP2.12.

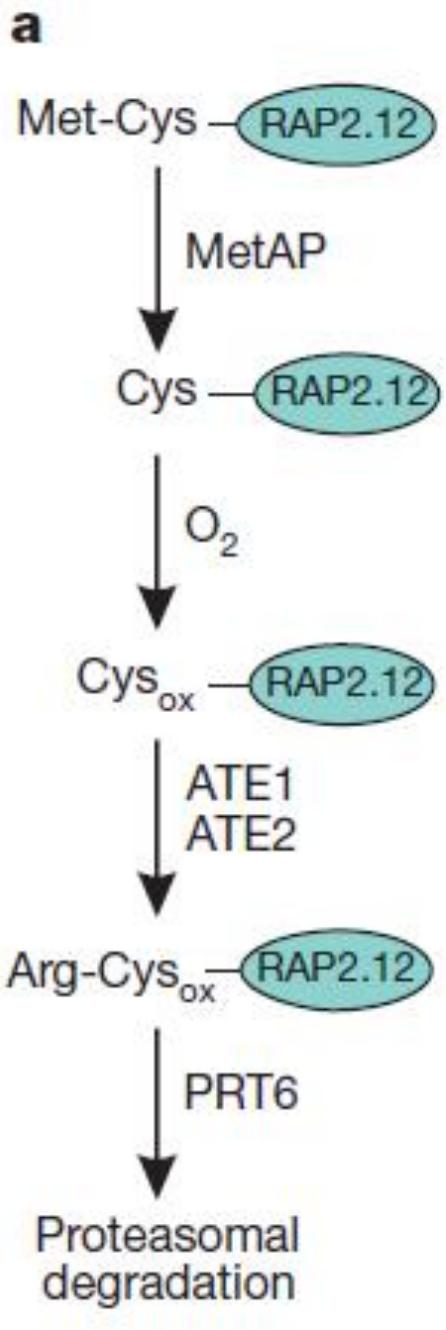
b, Yeast two-hybrid analysis showing interaction between RAP2.12 and ACBP1 and ACBP2

c, Bimolecular fluorescence complementation of YFP confirming interaction between RAP2.12 and ACBP1 and ACBP2.

d, Yeast two-hybrid analysis between various truncated RAP2.12 proteins and D30ACBP1.

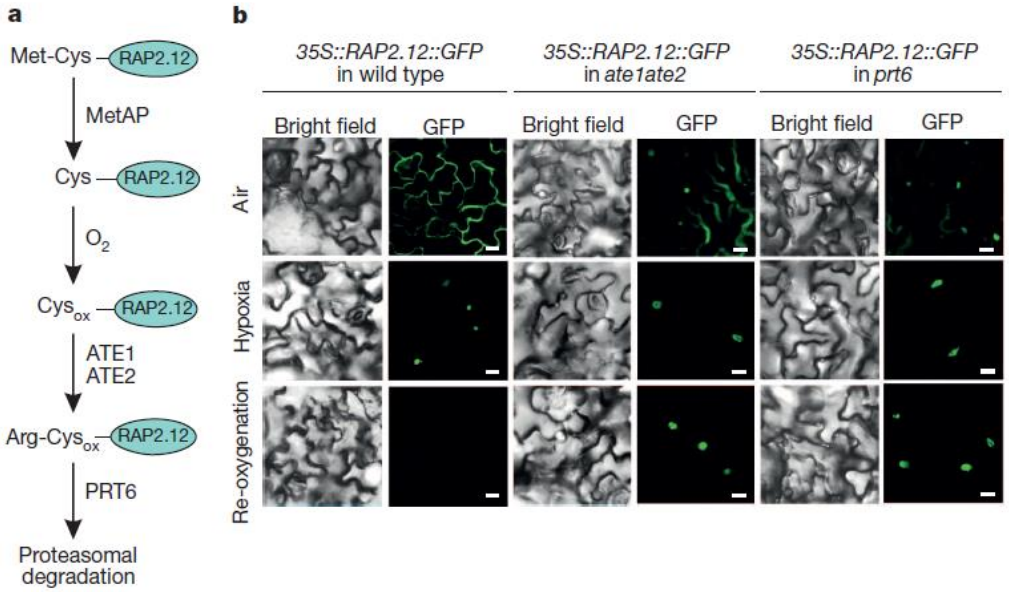
AD, activation domain; DBD, DNA-binding domain; UAS, upstream activator sequence.





Oxygen-dependent destabilization of RAP2.12

1. According to this pathway the terminal Met is removed from the protein by methionine aminopeptidase (MetAP) when the second amino acid of the protein is Cys
2. Terminal Cys is oxidized to cysteine sulphenic acid in an oxygen-dependent manner before arginine transferase (ATE) conjugates an Arg residue to the protein
3. This triggers subsequent ubiquitination by the ligase PROTEOLYSIS 6 (PRT6) and targets the protein to the proteasome for degradation



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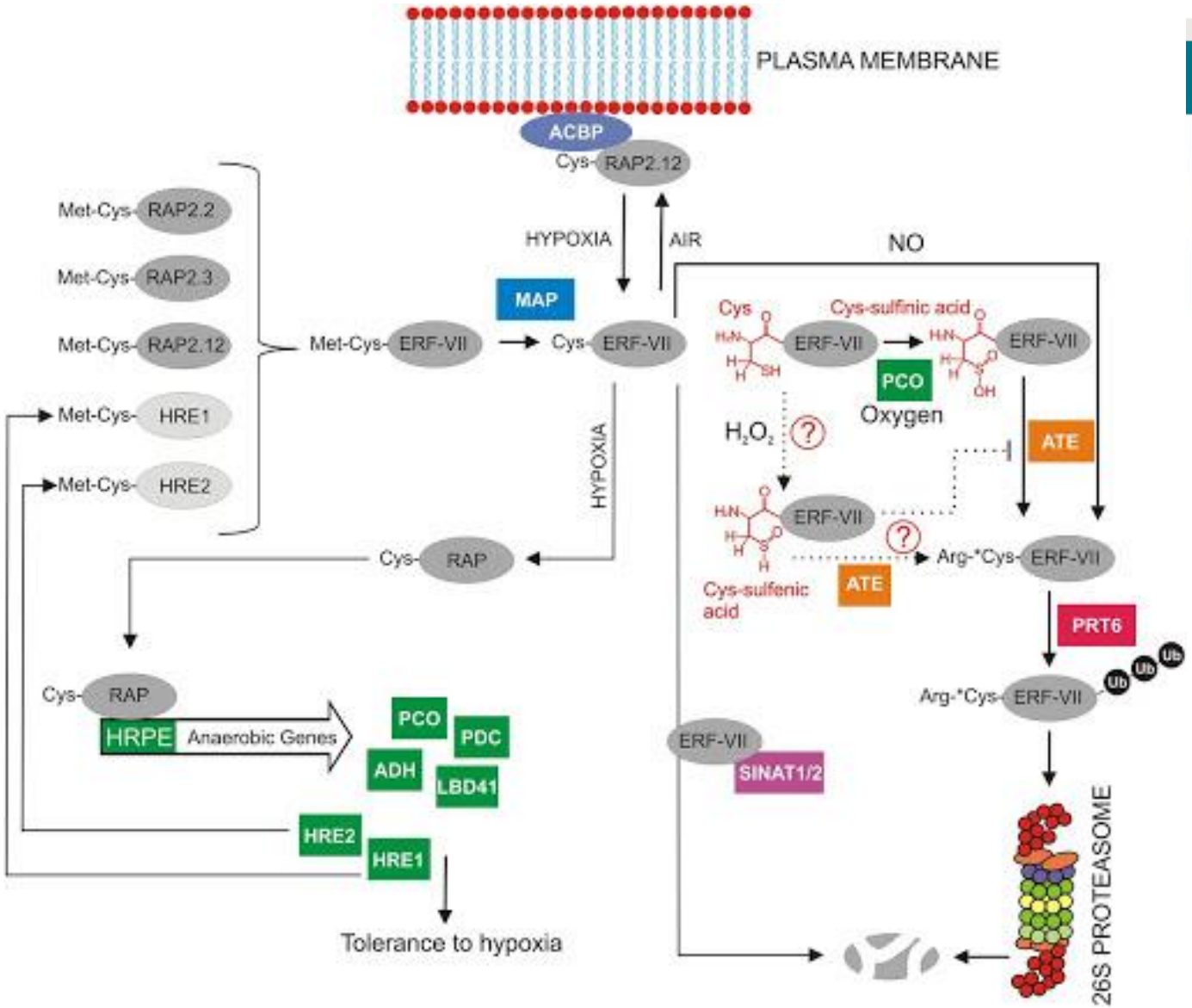
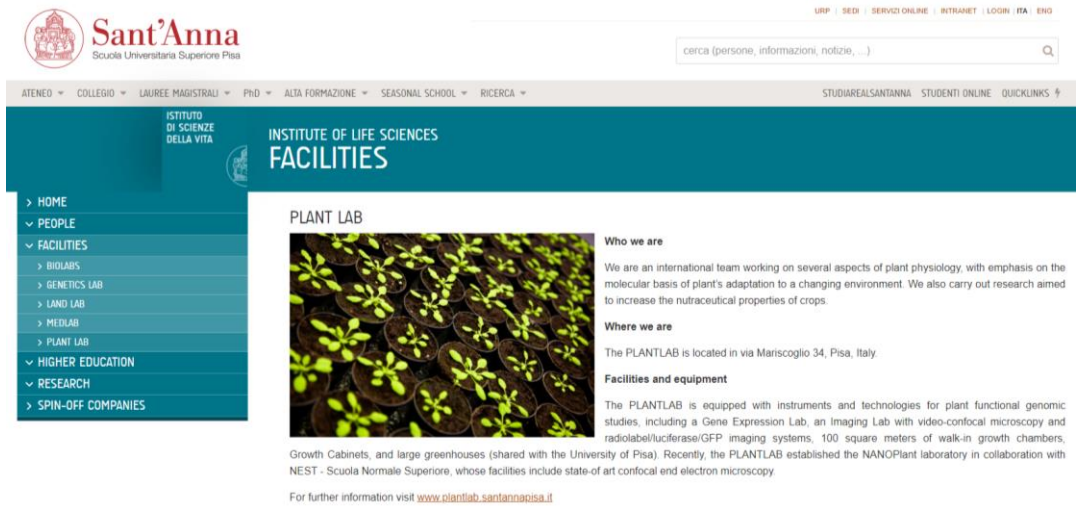

PLASMA MEMBRANE
ACBP
Cys-RAP2.12
HYPOXIA
AIR
NO
MAP
Met-Cys-RAP2.2
Met-Cys-RAP2.3
Met-Cys-RAP2.12
Met-Cys-ERF-VII
Cys-ERF-VII
Met-Cys-HRE1
Met-Cys-HRE2
HYPOXIA
Cys-RAP
Cys
Cys-sulfinic acid
ERF-VII
PCO
Oxygen
H_2O_2
ATE
Cys-sulfenic acid
Arg-*Cys-ERF-VII
PRT6
Ub
Cys-RAP
HRPE
Anaerobic Genes
PCO
PDC
ADH
LBD41
HRE2
HRE1
Tolerance to hypoxia
SINAT1/2
26S PROTEASOME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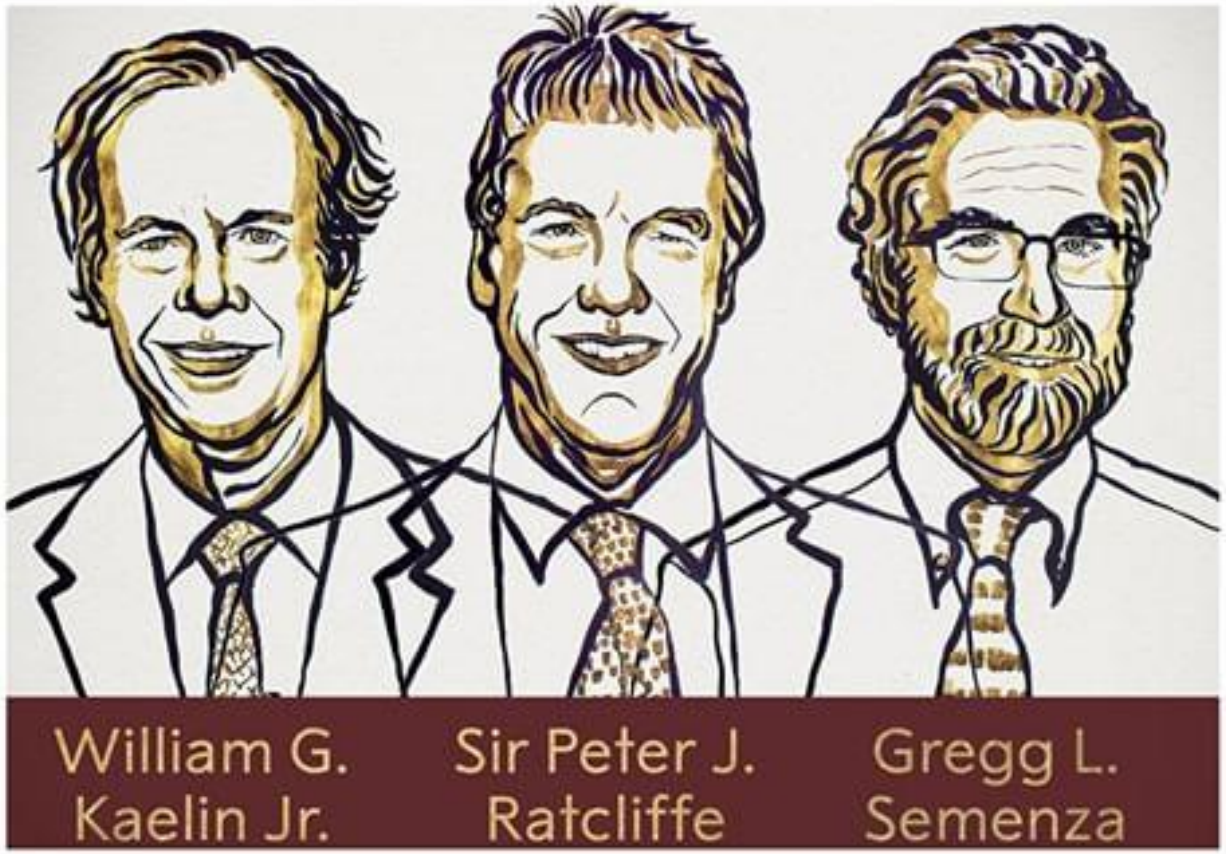
PLANT LAB
Who we are
We are an international team working on several aspects of plant physiology, with emphasis on the molecular basis of plant's adaptation to a changing environment. We also carry out research aimed to increase the nutraceutical properties of crops.
Where we are
The PLANTLAB is located in via Mariscoglio 34, Pisa, Italy.
Facilities and equipment
The PLANTLAB is equipped with instruments and technologies for plant functional genomic studies, including a Gene Expression Lab, an Imaging Lab with video-confocal microscopy and radiolabel/luciferase/GFP imaging systems, 100 square meters of walk-in growth chambers, Growth Cabinets, and large greenhouses (shared with the University of Pisa). Recently, the PLANTLAB established the NANOPlant laboratory in collaboration with NEST - Scuola Normale Superiore, whose facilities include state-of art confocal end electron microscopy.
For further information visit www.plantlab.santannapisa.it

- William G. Kaelin Jr.
- Sir Peter J. Ratcliffe
- Gregg L. Semenza

Nobel prize for medicine goes to scientists who found out how cells sense oxygen



MEDICINE PRIZE

Biologists who decoded oxygen sensing win Nobel

Laureates' discovery underpins understanding of diseases such as anaemia and cancer.

BY HEIDI LEDFORD & EWEN CALLAWAY

A trio of researchers has won the 2019 Nobel Prize in Physiology or Medicine for describing how cells sense and respond to changing oxygen levels by switching genes on and off — a discovery that has been key in understanding human diseases such as cancer and anaemia.

The three scientists are cancer researcher William Kaelin at the Dana-Farber Cancer Institute in Boston, Massachusetts; physician–scientist Peter Ratcliffe at the University of Oxford, UK, and the Francis Crick Institute in London; and geneticist Gregg Semenza at Johns Hopkins University in Baltimore, Maryland.

The team also won the Albert Lasker Basic Medical Research Award in 2016.

Their work has helped researchers to understand how the body adapts to low oxygen levels by, for example, cranking out red blood cells and growing new blood vessels.

"This is a fundamental discovery that they've contributed to," says Celeste Simon, a cancer biologist at the University of Pennsylvania in Philadelphia. "All organisms need oxygen, so it's really important."

"The field really coalesced around this discovery, which was dependent on each one of their findings," says Randall Johnson, a physiologist at the University of Cambridge, UK, and the Karolinska Institute in Stockholm, and a member of the Nobel Assembly. "This really was a three-legged stool."

L TO R: UNIV. OF OXFORD; HARVARD UNIV.; JOHNS HOPKINS MEDICINE

Nobel prizewinners Peter Ratcliffe (left), William Kaelin (centre) and Gregg Semenza (right).

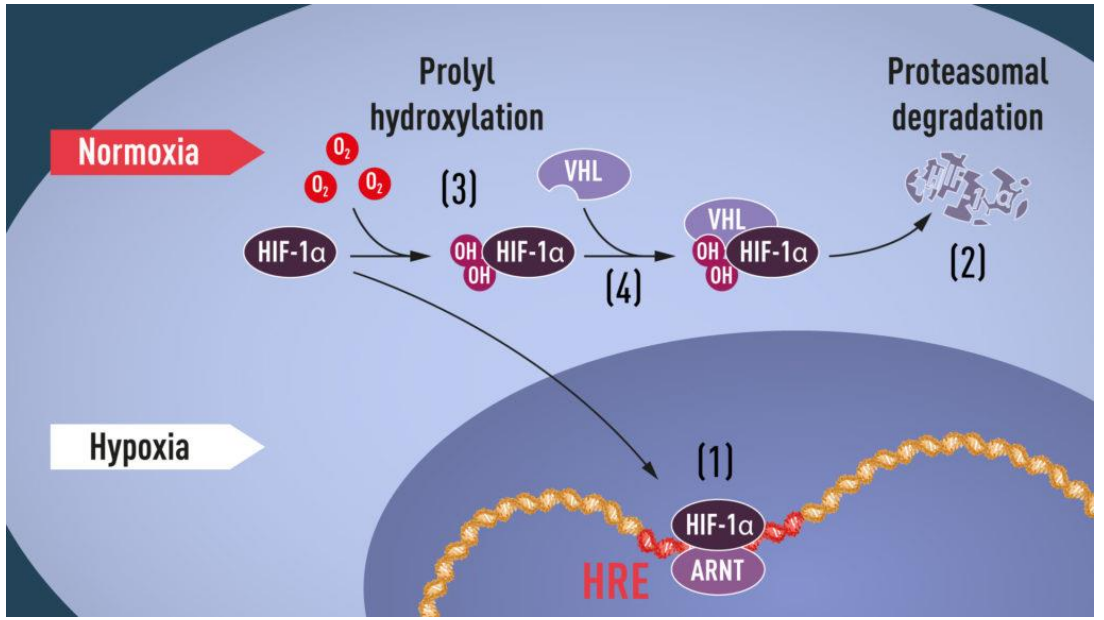
OXYGEN DEPRIVATION

The body's tissues can be deprived of oxygen during exercise or when blood flow is interrupted, such as during a stroke. Cells' ability to sense oxygen is also crucial for the developing fetus and placenta, as well as for tumour growth, because the mass of rapidly growing cells can deplete oxygen in a tumour's interior.

In work conducted in the 1990s, the scientists discovered the molecular processes that cells go through to respond to oxygen levels in the body. They found that central to this is a mechanism involving proteins called hypoxia-inducible factor (HIF) and VHL.

Semenza and Ratcliffe studied the regulation of a hormone called erythropoietin (EPO), which is crucial for stimulating the production of red blood cells in response to low oxygen levels. Semenza and his team identified a pair of genes that encode the two proteins that form the protein complex HIF, which turns on certain genes and boosts EPO production when oxygen is low.

Meanwhile, Kaelin showed that a gene called *VHL* also seemed to be involved in how cells respond to oxygen. Kaelin was studying a genetic syndrome called von Hippel-Lindau's disease; families with the disease carry mutations in *VHL*, and the condition raises the risk of certain cancers.



- Semenza discovered a protein complex he called "hypoxia-inducible factor" (HIF). Semenza further discovered that HIF is comprised of two transcription factors, now called HIF-1α and ARNT.
- Kaelin found that the VHL protein is needed to tag other proteins with ubiquitin. So without VHL the degradation of certain proteins is decreased, so their levels rise.
- Ratcliffe discovered that VHL interacts with HIF-1α, and is necessary for the degradation of HIF-1α at normal oxygen levels.

REVIEW SUMMARY

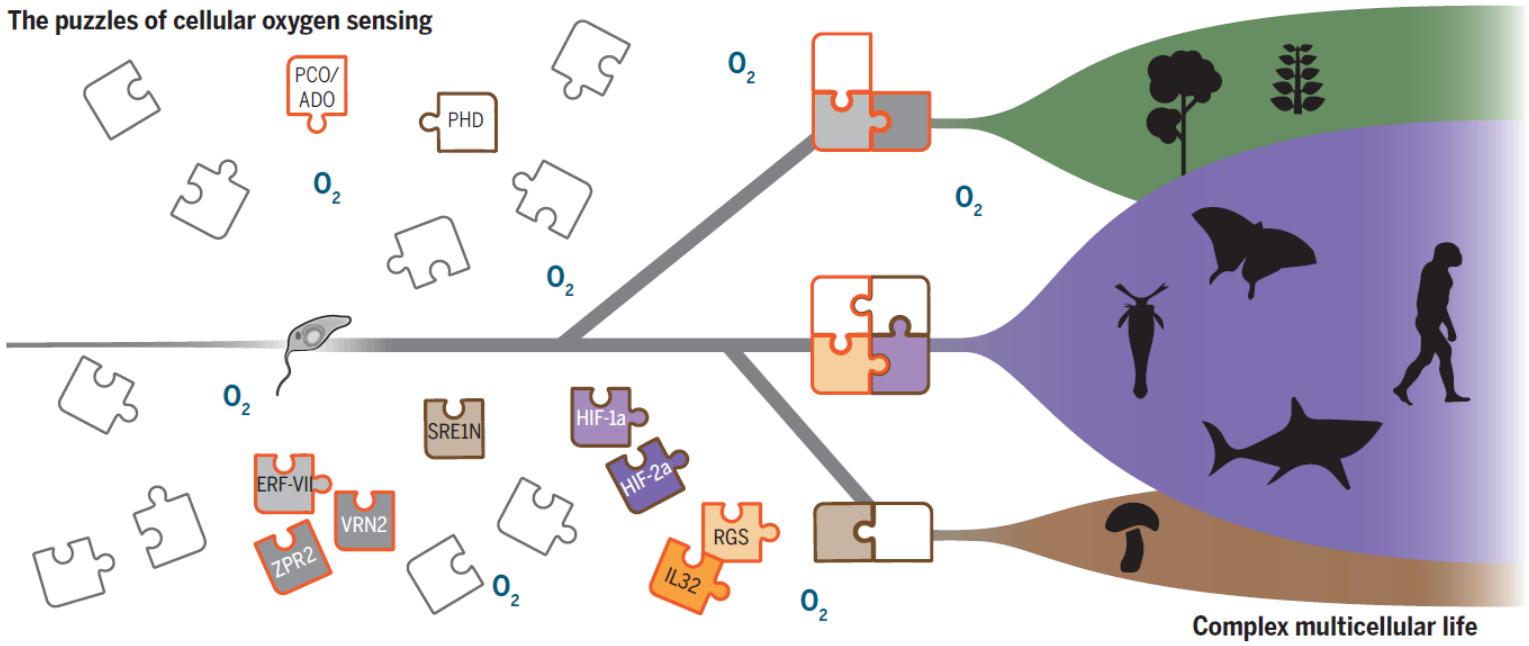
OXYGEN SENSING

Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity

Emma U. Hammarlund*†, Emily Flashman, Sofie Mohlin, Francesco Licausi*†

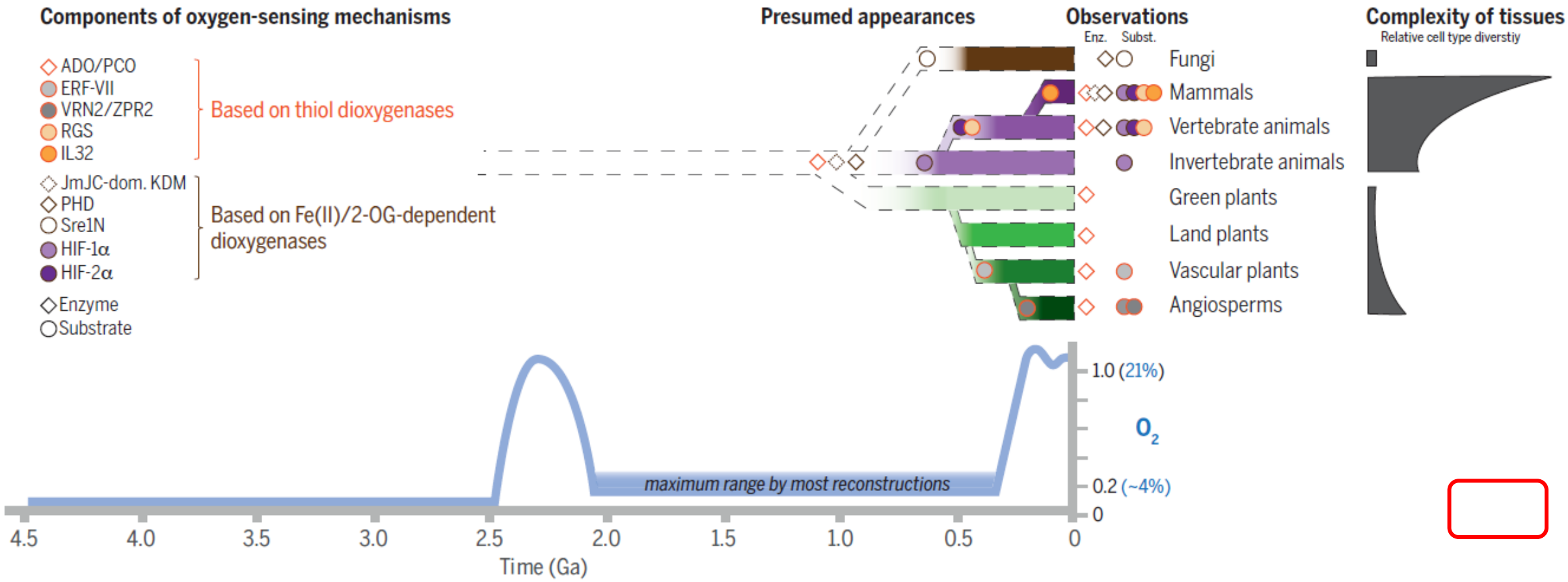
- Animals and land plants are the most **diverse** complex multicellular life-forms on Earth
- The performance of cell tasks, however, can be both dependent on and challenged by **oxygen**
- Oxygen acts as the final electron acceptor for **aerobic respiration** but also participates in reactions to **generate metabolites and structural macromolecules**
- Recently, oxygen also has come to the fore for its **signaling** role in developmental programs in animals and plants

For the rise of complex life, the capacity to link **oxygen perception to transcriptional responses** would have allowed organisms to attune cell fates to fluctuations in oxygen availability and metabolic needs in a spatiotemporal manner.



1. recruit dioxygenase enzymes to posttranslationally modify transcriptional regulators
2. proteasomal degradation at the relatively “normoxic” conditions
3. Transcriptional responses can be repressed at higher oxygen levels (which is context dependent) but are specifically elicited under hypoxia
4. the effects of prolonged hypoxia is also similar in animals and plants (transkingdom)

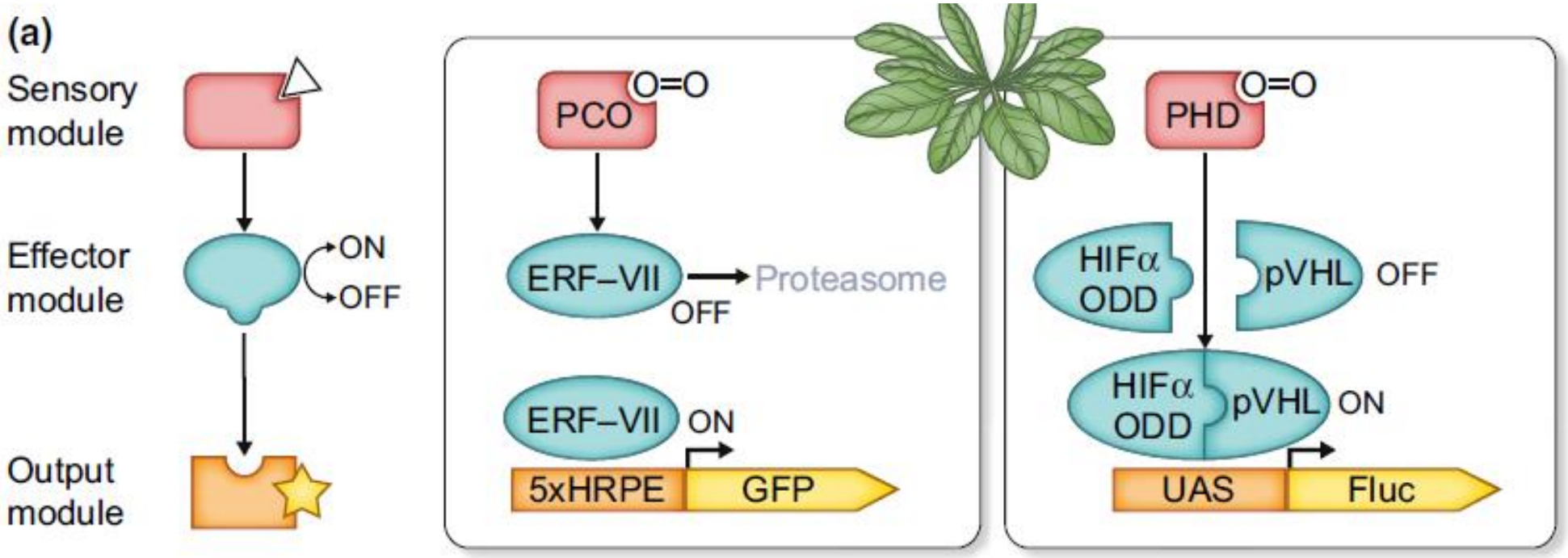
Increasing complexity of oxygen-sensing mechanisms and the extent of complexity within multicellular organisms over Earth's history of 4.6 Ga.



Enzymes (diamonds) and substrates (circles) form components of oxygen-sensing mechanisms, based on thiol dioxygenases (orange outlines) and Fe(II)/2-OG–dependent dioxygenases (brown outlines).

Reconstructions of atmospheric oxygen levels in the past. Eukaryotic kingdoms diversified (0.8 to 0.5 Ga ago), so the **evolution of oxygen-sensing mechanisms is rooted in hypoxic conditions**.
High atmospheric oxygen concentrations persisted at 2.5 to 2.0 Ga ago and then from 0.4 Ga ago (the Devonian Period) onward.

Oxygen sensing probes as future biotechnological application



Exploiting the Gal4/UAS System as Plant Orthogonal Molecular Toolbox to Control Reporter Expression in Arabidopsis Protoplasts

Sergio Iacopino, Francesco Licausi, and Beatrice Giuntoli

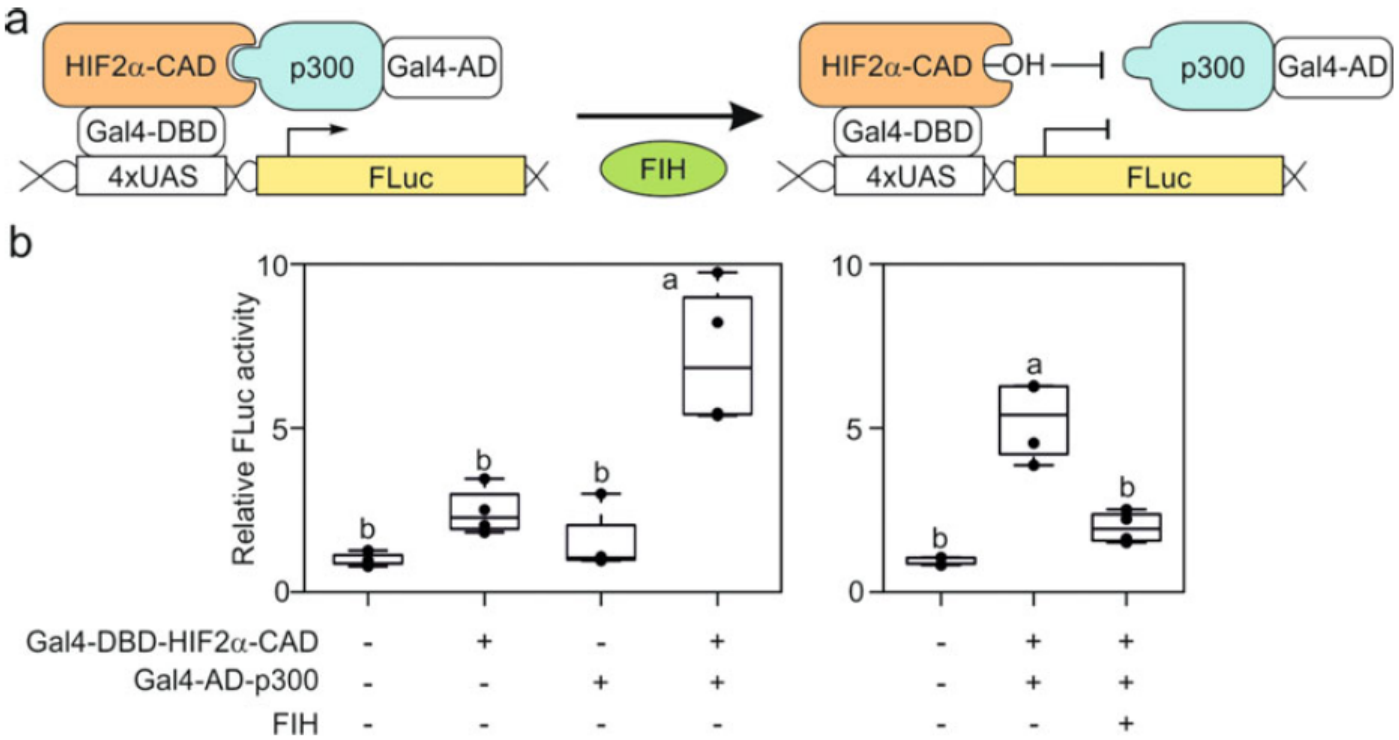


Fig. 1 Example of synthetic regulatory modules based on the GAL4/UAS system. (**a**) The C-terminal activation (CAD) domain of the HIF2α protein isolated from *Mus musculus* (aa 774–874) is fused with Gal4-DBD. The interaction with the TAZ type 1 domain of the human p300 protein (aa 300–528), in turn fused with Gal4-AD, triggers the formation of a heterodimeric transcription factor able to induce the expression of reporter genes located downstream of a promoter containing four repeats of the UAS sequence. Hydroxylation of Asparagine 851 by FIH hydroxylases hinders the interaction. (**b**) Comparison of FLuc activity among protoplasts transformed with the reporter plasmid, along with different combinations of effector plasmids depicted in (**a**). The output revealed an increase of FLuc activity exclusively when both Gal4 fused proteins were co-transformed together, in the absence of FIH

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